Karyotype Analysis in *Quercus* spp. (Fagaceae)

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Abstract

The karyomorphology of 8 species of *Quercus* has been studied. Chromosome number was 2n=2x=24 in all the taxa investigated. The karyotypes show great morphologic similarity, but individual species showed some differences of intrachromosomal and interchromosomal asymmetry indices. The species indicate a moderatly asymmetrical structure for the *Quercus* basic karyotype. *Q. dalechampii* and *Q. virgiliana* have slightly higher asymmetry level than the other species. Heteromorphic chromosome pairs were observed in some somatic metaphases of *Q. trojana, Q. crenata* and *Q. coccifera.*

Key words: Quercus, oak, Fagaceae, karyotype analysis, heteromorphic pair, taxonomy.

FDC: 165.3; 168; 176.1 *Quercus* spp..

Introduction

The genus *Quercus* includes about 300 species widespread from tropical and temperate forests to semi-arid zones of the Northern hemisphere. SCHWARZ (1964) grouped these species into 4 subgenera (*Schlerophyllodrys, Erythrobalanus, Cerris* and *Quercus*), but oak taxonomy is still debatable, despite various morphological, ecological, chemo-taxonomical and molecular studies (cf. KISSLING, 1980; KNOPS and JENSEN, 1980; AFZAL-RAFII, 1980; DUPOUEY, 1983; BIANCO and SCHIRONE, 1985; BELLAROSA et al., 1990; SCHIRONE et al., 1991; NIXON, 1993).

Cytological investigations on chromosome number in different oaks have also been conducted in order to clarify *Quercus* taxonomy (SAX, 1930; VIGNOLI, 1933; NATIVIDADE, 1937, DUFFIELD, 1940; TUTASUK and TURCHANINOVA, 1968; MEHRA et al., 1972; JOVANOVIC and TUCOVIC, 1975). However, the somatic number appears to be constant (2n=2x=24) for all species and does not prove useful in solving systematic and phylogenetic problems. Recently, OHRI and AHUJA (1990) described the karyotypes of 3 oak species, *Quercus robur* L., *Q. petraea* LIEBL. (subg. *Quercus*), and *Q. rubra* L. (subg. *Erythrobalanus*). These authors observed a general interspecific uniformity in oak karyotypes that complicated identification of the different species.

In the present paper, we report the results of the karyotype analyses of *Quercus cerris* L., *Q. trojana* WEBB, *Q. macrolepis* KOTSCHY and *Q. crenata* LAM., belonging to the subg. *Cerris; Q. frainetto* TEN., *Q. virgiliana* TEN. and *Q. dalechampii* TEN., belonging to the subg. *Quercus,* and *Q. coccifera* L., belonging to the subg. *Sclerophyllodrys.*

Material and Methods

Acorns of the different species were collected in various sites of Apulia (southern Italy), Latium (central Italy) and Sicily and germinated. Mitotic chromosomes were observed in actively growing root tips pretreated with 0.3% colchicine at room temperature for 2 h, and fixed for 5 min in an absolute alcohol, chloroform, glacial acetic acid, formalin mixture (5,1,1,1, volume ratio) (BATTAGLIA, 1957a). The root tips were hydrolyzed in concentrated HCl diluted 1:1 with distilled water for 20 min at room temperature (BATTAGLIA, 1957b). The material was then stained in freshly prepared Feulgen stain. Chromosomes of 5 to 6 metaphase plates were measured in 10 trees of each species.

Chromosome pairs were identified and arranged on the basis of chromosome length and arm ratio. For each oak species, the average apparent size of chromosomes and the ratio between the largest and the smallest (L/S) chromosomes were measured. Additionally, the karyotype asymmetry was evaluated calculating the centromeric index (I^C) and the parameters A_1 (intrachromosomic asymmetry index) and A_2 (interchromosomic asymmetry index) following ROMERO ZARCO (1986). Nomenclature recommended by LEVAN et al. (1964) for recognizing chromosome types was followed.

Table 1. – Chromosome numbers and karyotypic description of the examined oaks.

Species	Somatic number	Karyotypic description	Length range (μm)
Q. coccifera	2n=24	18m+2sm+4sm^{sc}	0.97-3.00
Q. crenata	2n=24	14m+2m^{sc}+6sm+2sm^{sc}	0.97-3.20
Q. cerris	2n=24	14m+4m^{sc}+4sm+2sm^{sc}	1.20-3.38
Q. macrolepis	2n=24	12m+4m^{sc}+8sm	1.22-3.57
Q. trojana	2n=24	8m+4m^{sc}+10sm+2sm^{sc}	1.05-3.02
Q. frainetto	2n=24	14m+2m^{sc}+6sm+2sm^{sc}	1.03-2.95
Q. dalechampii	2n=24	10m+2m^{sc}+8sm+4sm^{sc}	1.15-2.63
Q. virgiliana	2n=24	10m+4m^{sc}+8sm+2sm^{sc}	1.32-3.18

sc = secondary constriction

Table 2. – Morphometric parameters of the karyotypes of the *Quercus* spp. investigated. L/S = largest/shortest chromosome; I^c centromeric index; A_1 and A_2 (sensu ROMERO ZARCO, 1986). In parenthesis: standard error of mean.

Taxon	haploid complement μm	L/S	I^c	A_1	A_2
Subg. *Sclerophyllodrys*					
Q. coccifera	20.67 (± 1.07)	3.1 (± 0.29)	40.86 (± 0.30)	0.30 (± 0.01)	0.32 (± 0.02)
Subg. *Cerris*					
Q. crenata	20.72 (± 2.19)	3.3 (± 0.31)	40.82 (± 0.55)	0.30 (± 0.01)	0.37 (± 0.03)
Q. cerris	24.24 (± 0.91)	2.9 (± 0.21)	40.53 (± 0.63)	0.31 (± 0.02)	0.34 (± 0.02)
Q. trojana	20.67 (± 2.86)	2.9 (± 0.21)	40.44 (± 0.71)	0.31 (± 0.02)	0.35 (± 0.03)
Q. macrolepis	24.67 (± 1.41)	2.9 (± 0.09)	40.15 (± 0.50)	0.32 (± 0.01)	0.34 (± 0.01)
Subg. *Quercus*					
Q. frainetto	20.87 (± 0.94)	2.9 (± 0.36)	40.25 (± 0.52)	0.32 (± 0.01)	0.30 (± 0.03)
Q. virgiliana	25.00 (± 2.80)	2.5 (± 0.19)	39.00 (± 0.51)	0.35 (± 0.01)	0.26 (± 0.01)
Q. dalechampii	20.87 (± 1.47)	2.3 (± 0.16)	38.10 (± 0.39)	0.37 (± 0.01)	0.27 (± 0.01)

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Results

The chromosome number, 2n=2x=24, typical of the genus *Quercus,* was confirmed in each species. Chromosome numbers and karyotypes observed are reported in *table 1.* The morphometric parameters of the karyotypes of the species investigated are reported in *table 2.*

Somatic chromosomes of the investigated *Quercus* species were small and averages of chromosomal lengths ranged from 0.97 to 3.57 µm. Karyotype analyses reveals that the examined species were similar, but it was possible to observe some differences among karyotypes (*Tab. 1*) and individual chromosome morphology.

Q. cerris (*Figs. 1a, 2a*), *Q. macrolepis* (*Figs. 1b, 2b*), *Q. trojana* (*Figs. 3a, d*), and *Q. crenata* (*Figs. 3b, e*) have a secondary constriction on the short arm of a metacentric chromosome (pair 1), whereas *Q. coccifera* (*Figs. 1c, 2c*), *Q. virgiliana* (*Figs. 1d, 2d*), *Q. dalechampii* (*Figs. 1e, 2e*), and *Q. frainetto* (*Figs. 1f, 2f*) have a submetacentric chromosome pair 1 with a secondary constriction. *Q. coccifera* and *Q. cerris* karyotypes have a lower number of submetacentric chromosomes (6) with respect to the other species. *Q. dalechampii* and *Q. virgiliana* karyotypes were very similar and were characterized by ten metacentric and eight submetacentric chromosomes.

Generally there were 2 pairs of chromosomes with secondary constrictions in each karyotype. Additional secondary constrictions were observed in *Q. cerris, Q. trojana, Q. dalechampii* and *Q. virgiliana.* It was difficult to recognize, however, the satellited chromosomes, but exceptions with visible satellite have been observed in *Q. cerris* (*Figs. 1a, 2a*), *Q. crenata* (*Fig. 1g*) and *Q. virgiliana* (*Fig. 1h*). In *Q. coccifera, Q. crenata* and *Q. trojana* some metaphase plates showed a structural alteration in the chromosome pair 1 with an evident heteromorphy:

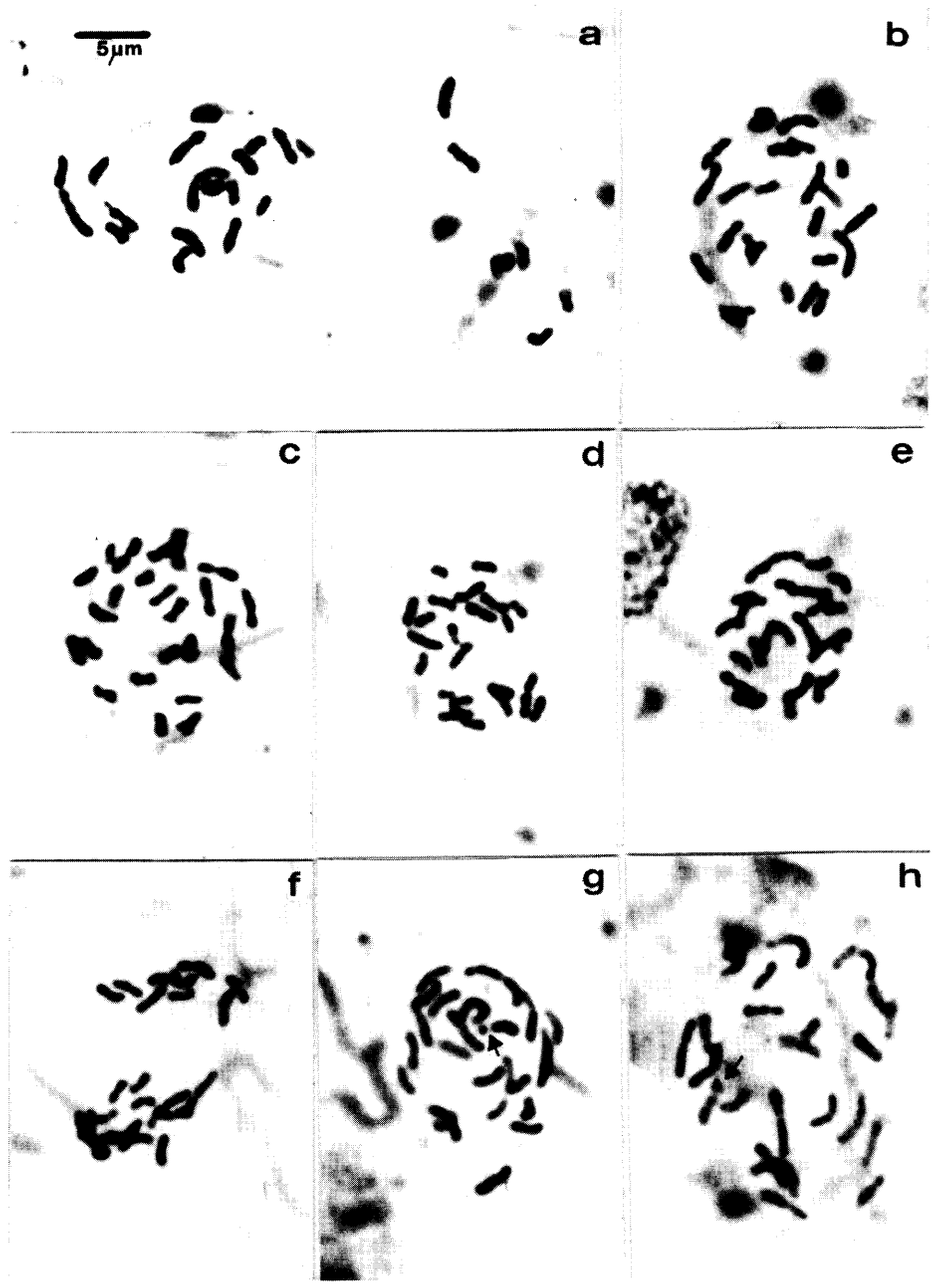

Figure 1. – Mitotic metaphases of a) *Q. cerris* L., b) *Q. macrolepis* KOTSCHY., c) *Q. coccifera* L., d) *Q. virgiliana* TEN., e) *Q. dalechampii* TEN., f) *Q. frainetto* TEN., g) *Q. crenata* LAM., h) *Q. virgiliana* TEN. Arrows in g,h indicate satellites.

one chromosome is submetacentric, the other metacentric (*Figs. 3a, b*).

The values for intrachromosomal asymmetry index (A_1) and interchromosomal asymmetry index (A_2) for each species were plotted in *figure 4*.

Discussion

The study again confirmed the consistency among *Quercus* species in having chromosome complement of 2n=24. The results also support the basic chromosome number of the genus as x=12, that concurs with most authors (cf. DUFFIELD, 1940; MEHRA et al., 1972; JONSSON and ERIKSSON, 1989; OHRI and AHUJA, 1990). Accessory chromosomes were not observed in this study. Occasionally, individual trees may exhibit ploidy variation as, e.g., 2 triploid specimens of *Q. robur* (with 2n=3x=36), recently found by BUTORINA (1993), and *Q. dentata* THUNB., in which contrasting results have been reported (48 somatic chromosomes in SAX, 1930, and n=12 and 2n=24 in SANTAMOUR, 1962). The polyploid specimens that sporadically appear in diploid populations are probably the result from meiotic nonreductions which producing diploid gametes (BRANDHAM, 1982; D'EMERICO et al., 1993). In an individual tree of *Q. frainetto* we observed, together with diploid cells, other cells containing endopolyploid plates with chromosome number 2n=4x=48 (*Fig. 3c*).

The majority of the examined species had karyotypes with predominance of metacentric chromosomes, excepting *Q. trojana, Q. dalechampii* and *Q. virgiliana* which usually show an equal sum of metacentric and submetacentric pairs.

Comparison of these karyotypes with those studied by OHRI and AHUJA (1990) shows close similarity in terms of number and morphology of metacentric and submetacentric pairs of chromosomes. In this study we did not find the subtelocentric pairs.

Analyses of the morphometric parameters in *table 2* revealed affinity between *Q. macrolepis, Q. frainetto, Q. trojana, Q. cer-*

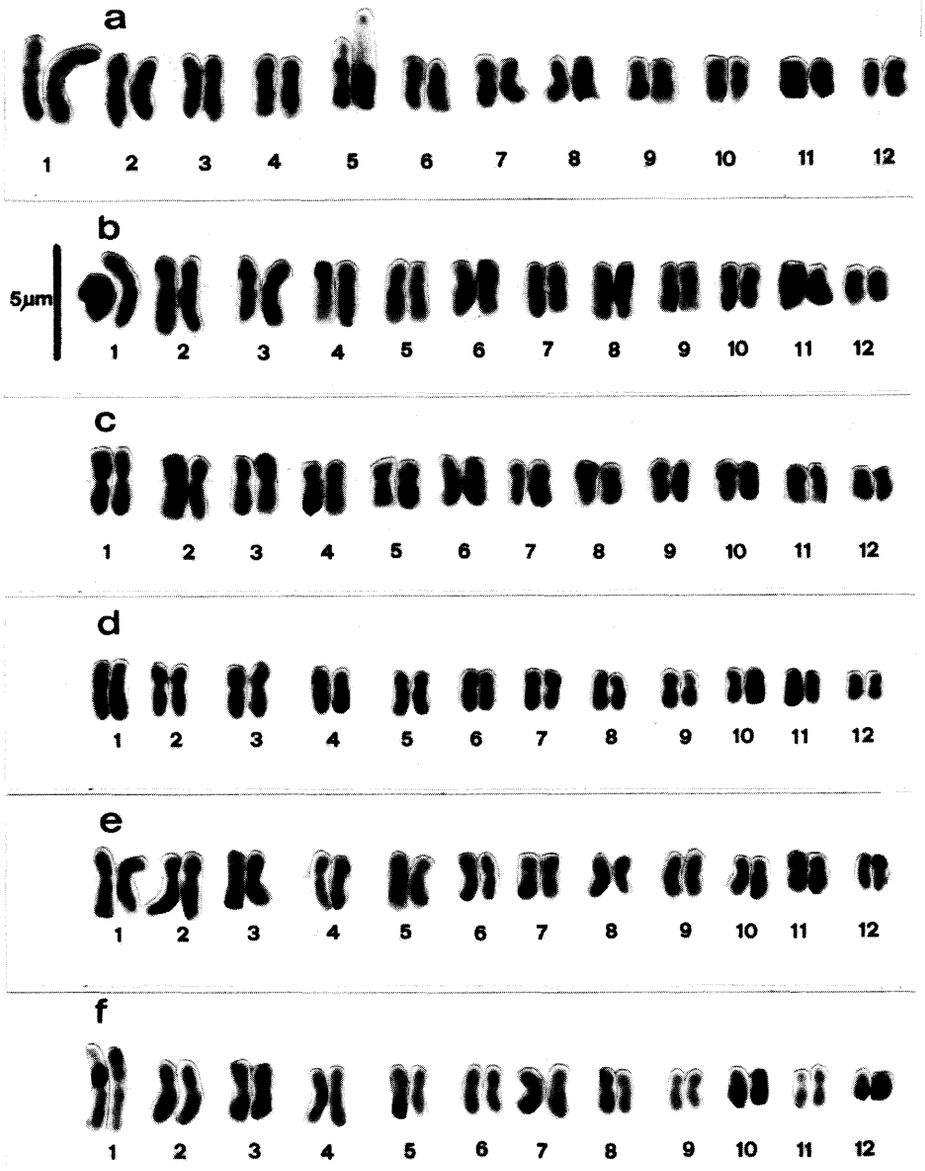

Figure 2. – Diploid karyotypes of a) *Q. cerris* L., b) *Q. macrolepis* KOTSCHY., c) *Q. coccifera* L., d) *Q. virgiliana* TEN., e) *Q. dalechampii* TEN., f) *Q. frainetto* TEN.

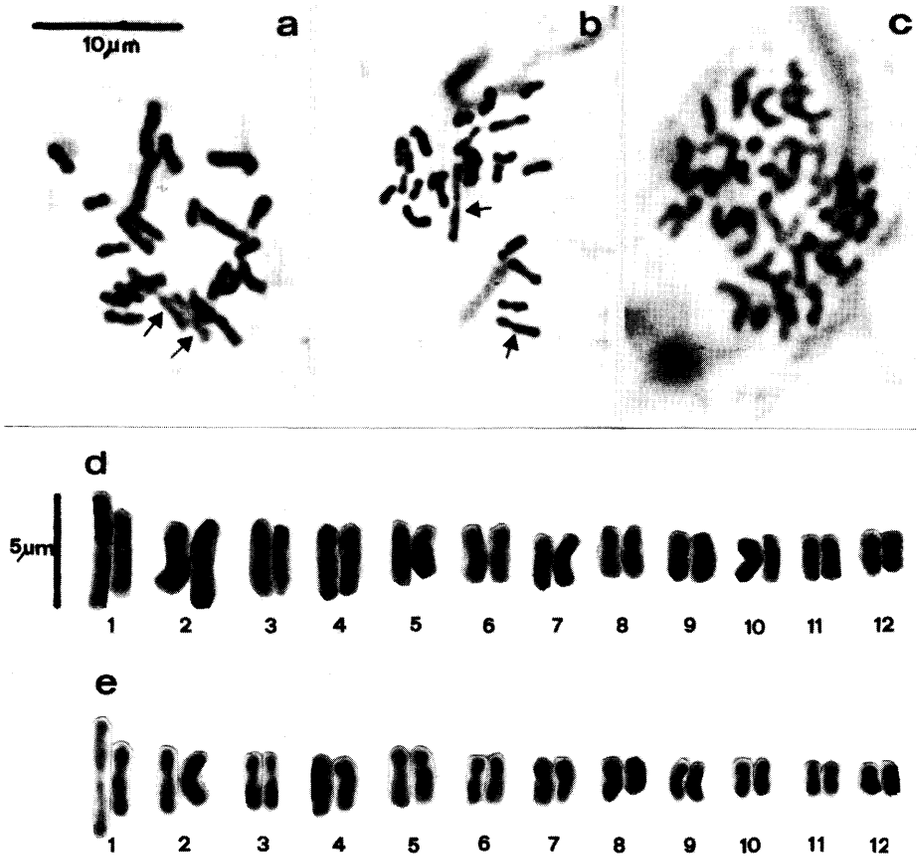

Figure 3. – Mitotic metaphase (a) and diploid karyotype (d) of *Q. trojana* WEBB; mitotic metaphase (b) and diploid karyotype (e) of *Q. crenata* LAM.; (c) mitotic metaphase 2n = 4x = 48 of *Q. frainetto*. Arrows in a,b indicate heteromorphic pair.

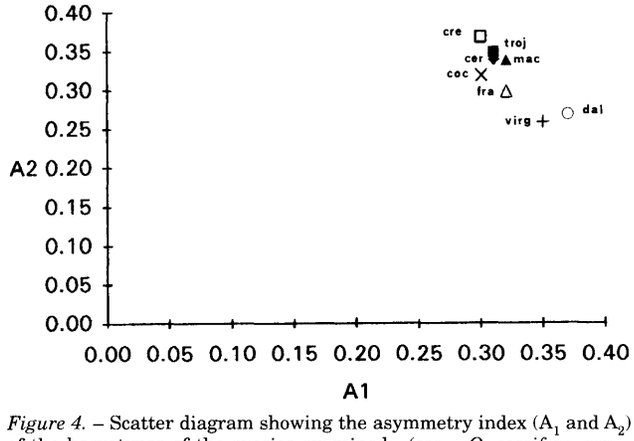

Figure 4. – Scatter diagram showing the asymmetry index (A_1 and A_2) of the karyotypes of the species examined. (coc = *Q. coccifera*, cer = *Q. cerris*, troj = *Q. trojana*, cre = *Q. crenata*, mac = *Q. macrolepis*, virg = *Q. virgiliana*, dal = *Q. dalechampii*, fra = *Q. frainetto*.

ris, Q. crenata and *Q. coccifera* karyotypes. They are similar for centromeric index (range 40.15 to 40.86) and for the ratio between the longest and the smallest (L/S) chromosomes (range 2.9 to 3.3). Moreover, the values for centromeric index (I^C) and intrachromosomal asymmetry index (A_1) indicate that the karyotypes of these species are slightly more symmetrical than those of *Q. dalechampii* and *Q. virgiliana*. The values obtained for the interchromosomal asymmetry index (A_2) are quite alike for *Q. cerris, Q. macrolepis* and *Q. trojana* karyotypes (range 0.34 to 0.35) and for those of *Q. dalechampii* and *Q. virgiliana* (range 0.26 to 0.27). These results indicate there is little variation in chromosomes size as to these last 2 species, in comparison with the ones belonging to subg. *Cerris*. It is interesting to note some relationships between karyotype characters and taxonomical position of the analyzed species. The scatter diagram in *figure 4* stresses the taxonomic affinity between *Q. cerris, Q. macrolepis* and *Q. trojana,* and the slightly higher asymmetry level of *Q. dalechampii* and *Q. virgiliana* in respect to the other species. Besides this, the diagram shows that *Q. frainetto* is separated from *Q. dalechampii* and *Q. virgiliana*. This might be seen when considering different morphological and ecological characters of *Q. frainetto* compared to *Q. dalechampii* and *Q. virgiliana* (ABBATE et al., 1987; MEDAGLI et al., 1990). However, all the species indicate a moderately asymmetrical structure for the *Quercus* basic karyotype, with symmetrical changes in some chromosome pairs resulting from rearrangements during evolution (STEBBINS, 1971). The relative homogeneity among *Quercus* karyotypes and constant ploidy level indicate that the species differentiation is primarily related to mutation of genes and chromosome rearrangements as suggested for the *Pinus* genus (PEDERICK, 1970; SAYLOR, 1983). The heteromorphy observed in some somatic metaphases of *Q. trojana, Q. crenata* and *Q. coccifera* might depend on structural rearrangements between chromosomes resulting from reciprocal translocations and inversions (CLAUSEN, 1967).

These results demonstrate that a comprehensive study involving karyotype analyses of many oak species may be useful in understanding *Quercus* taxonomy and evolution.

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References

ABBATE, G., BLASI, C., SPADA, F. and SCOPPOLA, A.: Analisi fitogeografica e sintassonomia dei querceti a *Quercus frainetto* TEN. dell'Italia centrale e meridionale. Not. Fitosoc. **24** (1987). — AFZAL-RAFII, Z.: Caractèristiques taxonomiques, morphologiques et isoenzymatiques du complex «Chene vert». Bull. Soc. Bot. France, 135. Lett. Bot. **14**/5: 342–352 (1988). — BATTAGLIA, E.: A new "5 minutes fixation" in chromosome analysis. Caryologia **9**: 368–370 (1957a). — BATTAGLIA, E.: A simplified Feulgen method using cold hydrolysis. Caryologia **9**: 372–373 (1957b). — BELLAROSA, R., DELRE, V., SCHIRONE, B. and MAGGINI, F.: Ribosomal RNA genes in *Quercus* spp. (*Fagaceae*). Pl. Syst. Evol. **172**: 127–139 (1990). — BIANCO, P. and SCHIRONE, B.: On *Quercus coccifera* L. s.l.: variation in reproductive phenology. Taxon **34**: 436–439 (1985). — BRANDHAM, P. E.: Inter-embryo competition in the progeny of autotriploid *Aloineae* (*Liliaceae*). Genetica **59**: 29–42 (1982). — BUTORINA, A. K.: Cytogenetic study of diploid and spontaneous triploid oaks, *Quercus robur* L. Ann. Sci. For. **50**: suppl. 1, 144s–150s (1993). — CLAUSEN, J.: Biosystematic consequences of ecotypic and chromosomal differentiation. Taxon **16**: 271–279 (1967). — D'EMERICO, S., BIANCO, P. and MEDAGLI, P.: Cytological and karyological studies on *Orchidaceae*. Caryologia **46**: 309–319 (1993). — DUFFIELD, W. J.: Chromosome counts in *Quercus*. Amer. J. Bot. **27**: 787–788 (1940). — DUPOUEY, J. L.: Analyse multivariable de quelques caractères morphologiques de populations de chenes (*Quercus robur* L. et *Quercus petraea* (MATT.) LIEBL.) DU HUREPOIX. Ann. Sci. For. **40**: 265–282 (1983). — JONSSON, A. and ERIKSSON, G.: Cytology, flowering frequency and hybridization in the genera *Acer, Fagus, Fraxinus, Prunus, Quercus, Tilia* and *Ulmus*. Dept. Forest Genetics, Swedish Univ., Agric. Sciences, Uppsala. Res. Note **43**, pp. 1–14 (1989). — JOVANOVIC, M. and TUCOVIC, A.: Genetics of common and sessile oaks (*Quercus robur* L. and *petraea* LIEBL.). Annales Forestales **7**: 23–53 (1975). — KISSLING, P.: Clé de détermination des chenes médioeuropéens (*Quercus* L.) Ber. Schweiz. Bot. Ges. **90**: 29–44 (1980). — KNOPS, J. F. and JENSEN, R.: Morphological and phenolic variation in a three species community of red oaks. Bull. Torrey Bot. Club **107**: 418–428 (1980). — LEVAN, A., FREDGA, K. and SANDBERG, A. A.: Nomenclature for centromeric position on chromosomes. Hereditas **52**: 201–220 (1964). — MEDAGLI, P., BIANCO, P., SCHIRONE, B., D'EMERICO, S. and RUGGIERO, L.: Il farnetto di Bosco Belvedere (Lecce). Note preliminari. Ann. Bot. (Roma) **XLVIII**, Supp. **7**: 77–83 (1990). — MEHRA, P. N., HANS, A. S. and SAREEN, T. S.: Cytomorphology of Himalayan Fagaceae. Silvae Genetica **21**: 102–109 (1972). — NATIVIDATE, J. V.: Investigacoes nalgumas especies e hybridos do genero *Quercus*. Bot. Soc. Brot. **12**: 21–85 (1937). — NIXON, KC.: Infrageneric classification of *Quercus* (*Fagaceae*) and typification of sectional names. Ann. Sci. For. **50**: 25–34 (1993). — OHRI, D. and AHUJA, M. R.: Giemsa C.banded karyotype in *Quercus* L. (Oak). Silvae Genetica **39**: 216–219 (1990). — PEDERICK, L. A.: Chromosome relationships between *Pinus* species. Silvae Genetica **19**: 171–180 (1970). — ROMERO ZARCO, C.: A new method for estimating karyotype asymmetry. Taxon **35**: 526–530 (1986). — SANTAMOUR, F. S. JR.: The chromosome number of *Quercus dentata*. Bull. Morris Arboret. **13**: 28–30 (1962). — SAX, H. J.: Chromosome numbers in *Quercus*. Arnold Arbor. **11**: 220–223 (1930). — SAYLOR, L. C.: Karyotype analysis of Genus *Pinus*-Subgenus *Strobus*. Silvae Genetica **32**: 119–124 (1983). — SCHIRONE, B., SCHIRONE, A., LOZUPONE, G., BELLAROSA, R. and GIORDANO, E.: Osservazioni sulle proteine di riserva del seme nel genere *Quercus*. Giorn. Bot. Ital. **125**: 231 (1991). — SCHWARZ, O.: *Quercus* L. In: Flora Europaea I. T. G. TUTIN et al. (eds.). Cambridge University Press, Cambridge. pp. 61–64 (1964). — STEBBINS, G. L.: Chromosomal evolution in higher plants. Edward Arnold Ltd., London (1971). — TUTASUK, V. H. and TURCHANINOVA, L. V.: About the chromosome number of *Quercus castaneifolia* C. A. M. Dokl. Akad. Nauk. Azerbajdzansk S. S. R. **24**: 47–50 (1968). — VIGNOLI, L.: Studio citologico sul genere *Quercus*. Lav. R. Ist. Bot. Palermo **4**: 25–39 (1933).

Altitudinal Adaptation of Norway Spruce (*Picea abies* (L.) Karst.) Progenies Indicates Small Role of Introduced Populations in the Karkonosze Mountains

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Summary

It is believed that many of the Norway spruce stands in Karkonosze Mts. are of foreign origin. To determine what is the real share of such stands, eighteen were ecotypically identified based on the phenology and growth of progeny. The characters, observed or measured in 2 distant nurseries were: bud set index (in the first and second growing season), bud flushing index (second growing season), diameter and height (after the second and fifth growing season) as well as height increment, crown diameter and roots dry matter (after the fifth growing period). It appears that most of the investigated stands fit well into the altitudes at present occupied by them in the Karkonosze Mts. Even the poorest adapted populations show average altitudinal deviations of 125 m to 156 m which fit easily the tolerance zones accepted for the seed transfer. It is strongly recomended that spruce stands in Karkonosze Mts. be regenerated in a natural way and that local seed resources be used for artificial regeneration.

Key words: Picea abies, ecotype identification, progeny characters, ecological adaptation, regeneration, West Sudety Mts.

FDC: 165.5; 181.2; 232.1; 174.7 *Picea abies*; (234.5); (438).

Introduction

There exists a belief, corresponding with the opinion of RUBNER (1936), that in Karkonosze Mts. ("Riesengebirge" in German) Norway spruce stands located above 1000 m elevation (upper mountain zone) are native and those located below (lower mountain zone) are of foreign origin. MATUSZKIEWICZ, W. and MATUSZKIEWICZ, A. (1967) as well as SOKOŁOWSKI (1968) write, however, that some spruce stands in the lower zone of these mountains are of natural, local origin. On the other hand ZOLL (1958) as well as PEŘINA and SAMEK (1958) suggest that numerous spruce stands in the upper zone of the Karkonosze Mts. have been established artificially, whith the use of imported seeds.